ACTION POTENTIAL TRANSFER AT THE PURKINJE - VENTRICULAR JUNCTION: ROLE OF TRANSITIONAL CELLS

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***Abstract* – At the Purkinje (P) - ventricular (V) junction a zone of "transitional (T)" cells is found. In the present study we investigated the role of these T cells in P-to-V conduction. Using the "model clamp" technique, an experimentally recorded rabbit P cell was coupled to a phase-2 Luo and Rudy (LR) model cell, which in turn was coupled to a strand of phase-2 LR model cells. In our experiments, the single LR model represents the T cell, while the strand of LR models represents subendocardial V cells. This approach enabled us to change selectively coupling conductance (G_c) between cells, presence of T cell, and relative size of cells. We demonstrated that: 1) a decrease of G_c between P-T and T-V increases the delay of V activation, 2) the delay of V activation is importantly due to conduction between T and V cells, 3) there is a critical G_c for successful conduction at the P-V junction, 4) the critical value of G_c for conduction at the P-V junction is lower in presence (11.0±0.7 nS) than in absence (13.7±0.8 nS) of the T cell, and 5) enlargement of the T zone size hampers successful P-to-V conduction.**

***Keywords* – Heart, electrophysiology, gap junction, Purkinje-ventricular junction, conduction**

I. INTRODUCTION

At the junction between Purkinje (P) fibers and ventricular (V) myocardium a tiny zone is found populated with cells that are called "transitional (T)" [1,2]. The P fibers are connected via short thin branches to sheets of these T cells, which in turn are connected via short thin branches to the V myocardium [3]. This anatomic arrangement is thought to form a high-resistance barrier [3], which may benefit P-to-V conduction by shielding a relatively small P fiber from the electrical load imposed by a relatively large V mass [4]. In addition, it is thought to contribute to the discontinuous conduction at the P-V junction [4,5]. In the present study, we measured the effects of the presence of T cells on action potential transfer at the P-V junction. Using an extended version of the "model clamp" technique [6], we electrically coupled an isolated rabbit cardiac P cell by any desired value of "gap junctional conductance" to a strand of ventricular cells of the phase-2 Luo and Rudy (LR) model [7]. In between, we can add another LR model cell, representing in our experimental set-up the T cell. This approach allowed us to study selectively the importance of T cells in conduction at the P-V junction.

II. METHODOLOGY

Cell preparation. Single P cells were isolated from rabbit hearts by enzymatic dissociation. Hearts were quickly removed from anaesthetized rabbits (1 ml/kg Hypnorm), mounted on a Langendorff perfusion apparatus, and perfused with the following solutions: 1) Tyrode's solution for 10 min; 2) nominally 'Ca^{2+}-free' Tyrode's solution for 10 min; and 3) nominally 'Ca^{2+}-free' Tyrode's solution with collagenase (59 U/L type B and 150 U/L type P; Boehringer) and 250 mg/L trypsin inhibitor (Boehringer) for 30 min. Subsequently, free-running P fibers, free from ventricular tissue, were excised from both ventricles and agitated in nominally 'Ca^{2+}-free' Tyrode's solution to obtain single P cells. All solutions were oxygenated and temperature was maintained at 36±1°C.

Electrophysiological recording. Using the whole-cell patch-clamp technique, action potentials were recorded from P cells in Tyrode's solution (36±1°C) containing (mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.5 glucose, and 5.0 HEPES (pH 7.4 with NaOH). Patch pipettes were pulled from borosilicate glass and filled with a solution containing (mM): 125 K-gluconate, 20 KCl, and 10 HEPES (pH 7.2 with KOH). Series resistance and pipette capacity was compensated up to ~80%. The majority of the enzymatically isolated P cells were quiescent, which agrees with many previous studies on single cardiac Purkinje cells [8, and primary refs. therein]. In all experiments, except one, we used quiescent P cells. Action potentials were elicited at 1 Hz (unless otherwise mentioned), and they were corrected for the estimated 13 mV change in liquid junction potential before starting the coupling experiments. Cell size was determined as we described in detail previously [6].

Model clamp technique. Real P cells were electrically coupled with a variable effective conductance (G_c) to a detailed mathematical model of a cardiac cell using the model clamp technique [6] (Fig. 1). In brief, the time-varying membrane potential of the real P cell (V_P) is fed into a 1-GHz AMD Athlon computer through the A/D converter of a data acquisition board (DigiData 1200, Axon Instruments). Next, a current input, $-I_c$, is computed and supplied dynamically through the D/A converter to the real P cell, to produce the effect of the interaction with the model cell, whereas the membrane potential of the model cell (V_M,) is computed with the current input for this cell, $+I_c$, as an additional ionic current to produce the effect of the interaction with the real P cell. The effective size of real cell was changed by a factor z_P by replacing the current input for this cell, $-I_c$, with $-I_c/z_P$. The size of the real P cell was thus normalized to the size of the model cell. We coupled an isolated P cell to the LR model, representing the T cell. This LR model in turn was coupled to a strand of 19 or 7 cells of the LR model, representing the subendocardial V cells. Fig. 2A, inset, shows a diagram of this geometry (not all subendocardial V cells are depicted). We have focused our experimental work with this technique mainly on the role of T cells in success or failure of conduction in situations of extreme uncoupling. For each determination, we use a 2-s period of uncoupling followed by 6-s of coupling at any desired value of G_c, which was followed by another 2-s period of uncoupling.

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Abstract

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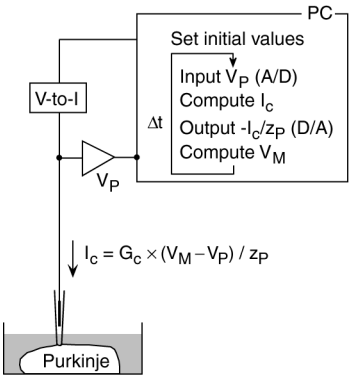

Fig. 1. Model clamp technique. The membrane potential of the real P cell, V_P, is recorded using an amplifier in the current clamp mode, and sampled into a microcomputer (PC). The coupling current, I_c, is computed according to $I_c = G_c \times (V_M - V_P)$, and a command voltage for the V-to-I converter is generated such that a current input $-I_c/z_P$ is supplied to the real P cell. z_P is a factor by which the effective size of the P cell can be changed. G_c is the coupling conductance, which can be varied over a wide range. The membrane potential of the model cell, V_M, is computed from the mathematical model with the current input for this cell, $+I_c/z_M$, as an additional ionic current.

III. Results

Effects of low G_c around T cells. The zone with T cells is thought to form a high-resistance barrier [3], which favors P-to-V conduction by shielding the relatively small P fiber from the electrical load imposed by a relatively large V mass [4]. In a first experiment, we tested this hypothesis. Fig. 2 illustrates the effects of low G_c around the T cell on conduction at the P-V junction. P cell size was normalized to the size of the LR model, and G_c within the strand of 19 LR model cells was fixed at a relatively high value of 50 nS to simulate a large V mass. G_c between P-T and T-V was symmetrized and varied in the range from 6 to 24 nS (Fig 2, insets). At $G_c \leq 6$ nS, conduction failed from P-to-T (Fig. 2A). At $G_c \geq 8$ nS, conduction from P-to-T succeeded, but conduction from T-to-V failed (Fig. 2B). Conduction from P-to-T-to-V was present at $G_c \geq 20$ nS (Figs. 2C and 2D). It must be noted that in case of successful P-to-T-to-V conduction, increasing G_c required much higher current pulses for eliciting P action potentials.

Effects of presence of T cells on critical G_c. In the typical example of Fig. 2, the lowest value of G_c for which action potentials during the coupling period were successfully conducted to the strand of V cells was 20 nS. This value was defined as the critical value of G_c for propagation at the P-V junction. In a second experiment, we analyzed the role of the T cell on this critical G_c. Therefore, action potential transfer was measured in presence and absence of the T cell. Fig. 3 shows a typical example. The size of the P cell was equal to the size of the LR model cell, and the G_c between all cells was varied in a range of 10 to 15 nS. When the T cell was present, conduction from P-to-T-to-V succeeded at $G_c \geq 11$ nS (Figs. 3A and 3C). When the T cell was absent, conduction from P-to-V failed at G_c of 11 nS (Fig. 3B), but succeeded at $G_c \geq 13$ nS (Fig. 3D). Similar effects were observed in another two experiments. The average critical value of G_c was 13.7±0.8 and 11.0±0.7 nS (n=3; $P \leq 0.05$, *t*-test) in absence and presence of the T cell, respectively. In one experiment, we tested the effect of stimulus frequency on conduction in absence and presence of T cells. In this experiment, the P cell size was equal to the LR model size, and G_c between all cells was 12 nS. In the complete stimulus frequency range tested (2.0, 1.0, 0.67, and 0.5 Hz), action potentials were successfully conducted to the V cells in presence, but never in absence of the T cell (data not shown).

Effects of T cell size. In a final experiment, we tested the effects of the size of T cells on conduction at the P-V junction. Fig. 4 illustrates the role of T cell size in case of a spontaneously active P cell. The P cell was coupled to the T cell, which in turn was coupled to a strand of 7 V cells. P cell size was 20 times that of the LR model. G_c between P-T was 20 nS, while that between T-V and within the strand was 10 nS. Action potential propagation from P-to-T-to-V succeeded

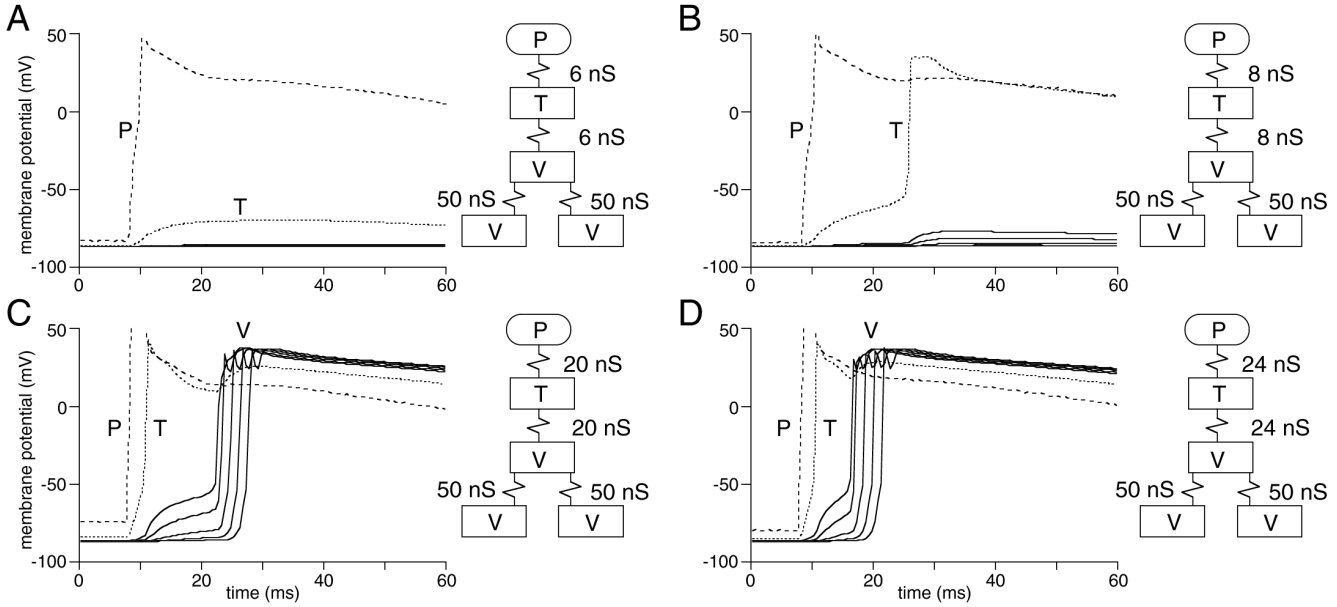

Fig. 2. Effect of low G_c around T cell for P-V conduction. **A, B, C, and D:** P cell is coupled to a T cell, which in turn is coupled to a strand of 19 V cells. Within the strand, cells were coupled at a fixed G_c of 50 nS. G_c between P-T and T-V were 6 nS (A), 8 nS (B), 20 nS (C), and 24 nS (D).

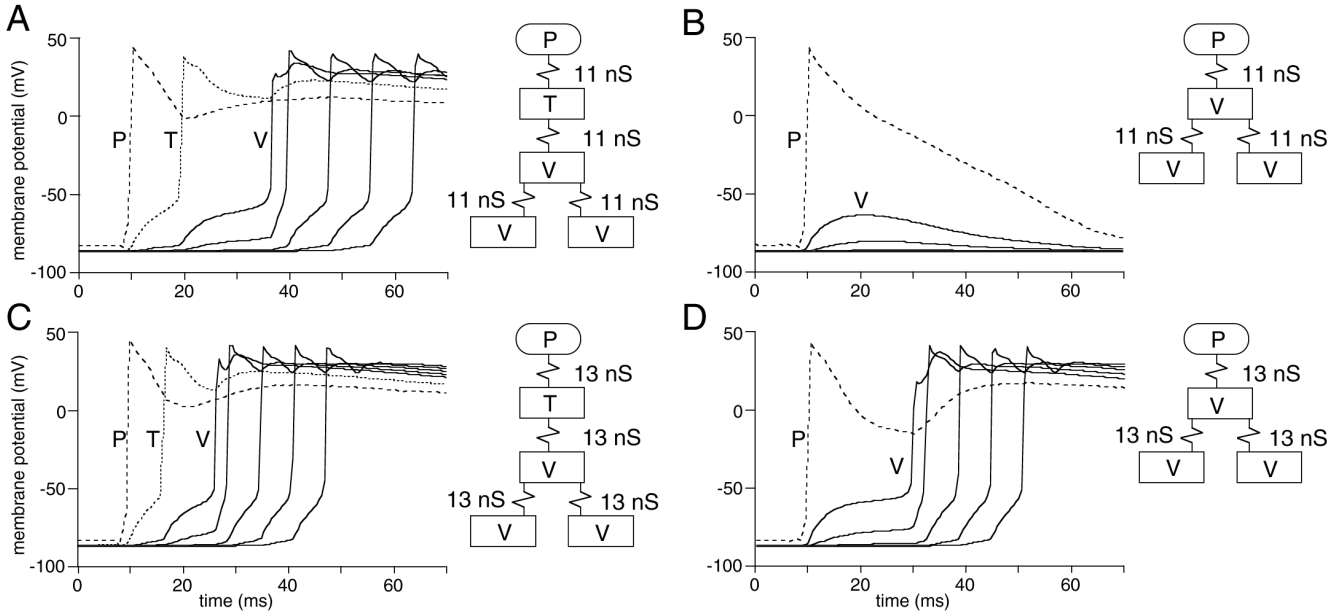

Fig. 3. Effects of T cell on critical G_c. **A, and B:** Cells were coupled at a G_c of 11 nS in presence (A) and absence (B) of T cell. Strand contained 19 V cells **C, and D:** Cells were coupled at a G_c of 13 nS in presence (C) and absence (D) of T cell. Strand contained 19 V cells.

when the T cell size was 2 times that of V cells (Fig. 4A), but failed when it was 5 times that of the V cells (Fig. 4B). Comparable effects were observed when we used stimulated cells. In the typical example of Fig. 4, action potential duration was decreased under both conditions of successful and failing action potential transfer (Fig. 4C), while membrane oscillations, due to early afterdepolarizations (EADs), were abolished. In addition, the phase of diastolic depolarization was prolonged (Fig. 4D), but the frequency was not changed much (Fig. 4C).

IV. Discussion

The aim of the present study was to investigate the role of T cells in action potential transfer at the P-V junction. Using the model clamp technique, we coupled a real rabbit cardiac P cell to a single LR model cell, which in turn was coupled to a strand of LR model cells. The single LR model represents in our experiments a T cell, while the strand of LR model cells represents a layer of subendocardial V cells. The present study thus extends the previous studies of Huelsing et al. [9,10,11] and Verkerk et al. [12] to conditions more closely representing those of the anatomically complex P-V junction. In those previous studies, the "analog coupling clamp" technique was used to study interactions between one real P cell and one real V cell [9,10] or a real P cell, with or without EADs, coupled to a resistance-capacitance circuit, which represented a passive V cell [11,12].

Cell models. In our experiments, we used the LR model of

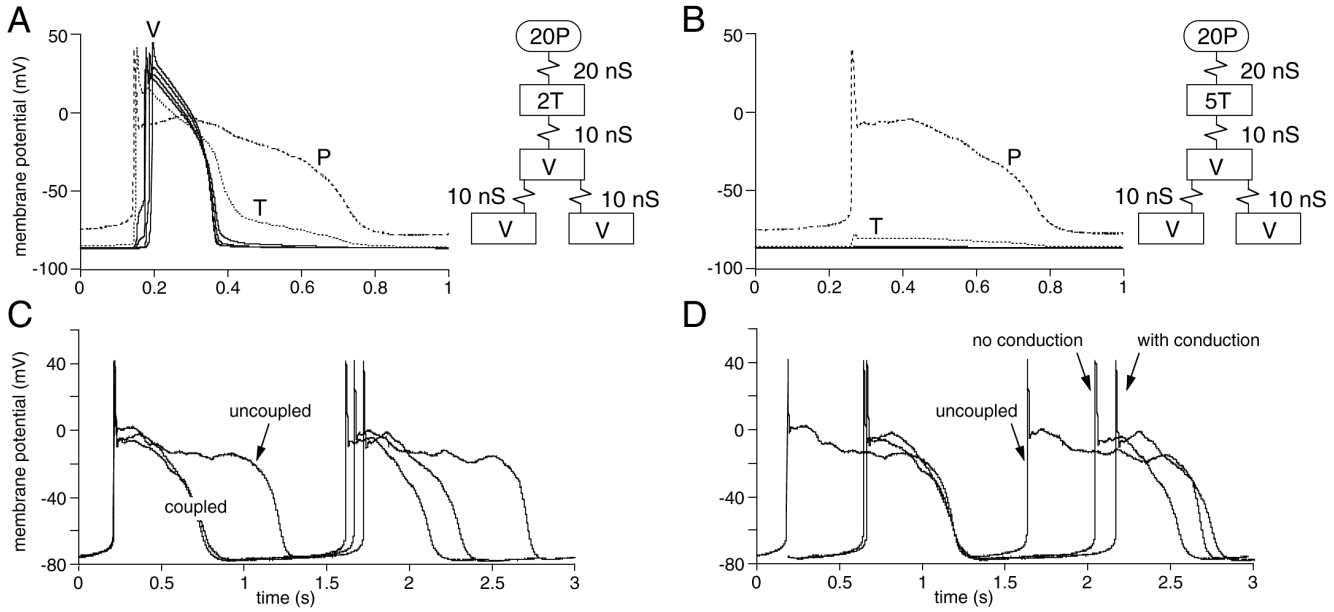

Fig. 4. Effects of T cell size on conduction at P-V junction. **A, and B:** P cell is coupled to a T cell, which in turn is coupled to a strand of 7 V cells. Size of the T cell is 2 (A) and 5 (B) times the size of the V cell to which it is connected. **C, and D:** Effects of coupling on action potential duration and EADs (C), and diastolic depolarization phase (D) of the P cell. Depicted are the P action potentials of panel A and B, and those recorded under uncoupled conditions.

a guinea pig V cell to simulate the T cell. Using microelectrodes, it was found that T cells have several electrophysiologic parameters intermediate between those of P fibers and the V myocardium [1]. These electrophysiologic characteristics, however, are largely variable, most likely due to a heterogeneous coupling between P fibers and V myocardium [3]. This coupling and the extremely thin zone of T cells make exact determination of electrical properties of T cells impossible. In addition, we used the LR model to simulate subendocardial V cells. The LR model has no transient outward K^+ current comparable to subendocardial V cells of many species.

EADs and spontaneous activity. We found that EADs were abolished in the real P cell upon coupling to a detailed cardiac cell model (Fig. 4). This effect is comparable to previous studies, where real cells showing EADs were coupled to a passive RC circuit [11-14]. In our experiments, however, we additionally demonstrated that this occurs under conditions of failing as well as successful action potential propagation (Fig. 4C). In addition, we demonstrated that the diastolic depolarization rate decreased upon coupling and this effect was found under both conditions of failing and successful action potential propagation (Fig. 4D). This effect is comparable to coupling a spontaneously active sinoatrial node model cell to a real atrial cell [15], and is most likely due to the electrotonic load of the transitional cell.

Role of T cells. The zone with T cells is thought to form a high-resistance barrier between the P fibers and the V mass, which may favor P-to-V conduction [3,4]. In the present study, we focused mainly on the role of the T cell in success or failure of conduction in situations of extreme uncoupling. We demonstrated that: 1) a decrease of G_c between P-T and T-V increases the delay of V activation (Figs. 2C and 2D), 2) the delay of V activation is importantly due to conduction between the T cell and the strand of V cells (Figs. 2A, 2B, and 3A), 3) there is a critical G_c for successful conduction at the P-V junction (Fig. 2), 4) the critical value of G_c for conduction at the P-V junction is lower in presence (11.0±0.7 nS) than in absence (13.7±0.8 nS) of the T cell, and 5) enlargement of the T zone size hampers successful P-to-V conduction (Figs. 4A and 4B).

V. Conclusion

In situations of extreme uncoupling, the presence of the T cell favors P-to-V conduction. The conduction delay at P-V junction is importantly due to action potential transfer between T and V cells.

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References

[1] K. Matsuda, "Some electrical properties of terminal Purkinje fibers of the heart," in *Electrical Activity of Single Cell*, Y. Katsuki, Ed. Tokyo: Igakushoin, 1960, pp. 283-294.

[2] K. Matsuda, A. Kamiyama, and T. Hoshi, "Configuration of the transmembrane action potential of the Purkinje-ventricular fiber junction and its analysis," in *Electrophysiology and Ultrastructure of the Heart*, T. Sano, V. Mizuhira, and K. Matsuda, Eds. New York: Grune and Stratton, 1967, pp. 177-187.

[3] J. Tranum-Jensen, A.A.M. Wilde, J.T. Vermeulen, and M.J. Janse, "Morphology of electrophysiologically identified junctions between Purkinje fibers and ventricular muscle in rabbit and pig hearts," *Circ. Res.,* vol 69, pp. 429-437, 1991.

[4] R.W. Joyner, R. Veenstra, D.A. Rawling, and A. Chorro, "Propagation through electrically coupled cells: effects of a resistive barrier," *Biophys. J.*, vol 45, pp. 1017-1025, 1984.

[5] R.T. Wiedmann, R.C. Tan, and R.W. Joyner, "Discontinuous conduction at Purkinje-ventricular muscle junction," *Am. J. Physiol.*, vol 271, pp. H1507-H1516, 1996.

[6] R. Wilders et al., "Action potential conduction between a ventricular cell model and an isolated ventricular cell," *Biophys. J.*, vol 70, pp. 281-295, 1996.

[7] C.H. Luo and Y. Rudy, "A dynamic model of the cardiac ventricular action potential, I: Simulations of ionic currents and concentration changes," *Circ. Res.*, vol. 74, pp. 1071–1096, 1994.

[8] A.O. Verkerk et al., "Two types of action potential configurations in single cardiac Purkinje cells of sheep," *Am. J. Physiol.*, vol 277, pp. H1299-H1310, 1999.

[9] D.J. Huelsing, K.W. Spitzer, J.M. Cordeiro, and A.E. Pollard, "Conduction between isolated rabbit Purkinje and ventricular myocytes coupled by a variable resistance," *Am. J. Physiol.*, vol 274, pp. H1163–H1173, 1998.

[10] D.J. Huelsing, K.W. Spitzer, J.M. Cordeiro, and A.E. Pollard, "Modulation of repolarization in rabbit Purkinje and ventricular myocytes coupled by a variable resistance," *Am. J. Physiol.*, vol 276, pp. H572–H581, 1999.

[11] D.J. Huelsing, K.W. Spitzer, and A.E. Pollard, "Electrotonic suppression of early afterdepolarization in isolated rabbit Purkinje myocytes," *Am. J. Physiol.*, vol 279, pp. H250–H259, 2000.

[12] A.O. Verkerk, M.W. Veldkamp, R. Coronel, R. Wilders, and A.C.G. van Ginneken, "Effects of cell-to-cell uncoupling and catecholamines on Purkinje and ventricular action potentials: implications for phase-1B arrhythmias," *Cardiovasc. Res.,* in press.

[13] R. Kumar, and R.W. Joyner, "An experimental model of the production of early afterdepolarizations by injury current from an ischemic region," *Pflügers Arch.*, vol 428, pp. 425-432, 1994.

[14] A.O. Verkerk, M.W. Veldkamp, N. de Jonge, R. Wilders, and A.C.G. van Ginneken, "Injury current modulates afterdepolarizations in single human ventricular cells," *Cardiovasc. Res.*, vol 47, pp. 124-132, 2000.

[15] R.W. Joyner et al., "Electrical interactions between a rabbit atrial cell and a nodal cell model," *Am. J. Physiol.*, vol 274, pp. H2152-H2162, 1998.

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